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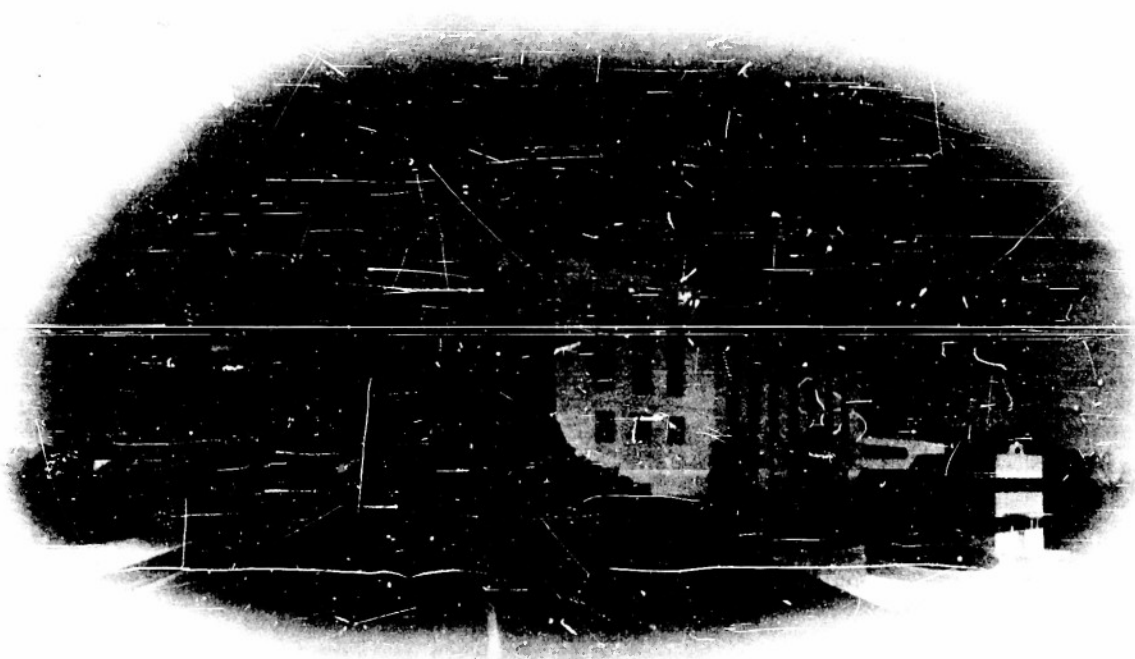
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A HIGH-IMPEDANCE INPUT CIRCUIT SUITABLE FOR ELECTROPHYSIOLOGICAL
RECORDING FROM MICROPIPETTE ELECTRODES

RESEARCH REPORT
Project NM 000 019.03.01

**A HIGH-IMPEDANCE INPUT CIRCUIT SUITABLE FOR ELECTROPHYSIOLOGICAL
RECORDING FROM MICROPIPETTE ELECTRODES**

E. F. MacNICHOL, Jr.
Thomas C. Jenkins Laboratories of Biophysics
The Johns Hopkins University, Baltimore, Maryland

HENRY G. WAGNER
Commander, MC, USN

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ABSTRACT

Recent interest in the use of intracellular microelectrodes for recording the electrical potential across a cell membrane has introduced a number of technical problems for solution. One of these problems concerns the development of special input devices that can be inserted between micropipette electrodes and conventional physiological amplifiers. This report discusses circuits in which a very high input impedance can be achieved without loss in speed of response by the use of both positive and negative feedback principles.

A preamplifier that has been constructed using these principles is described in considerable detail. It has been in use for about a year in neurophysiological investigation and has served to demonstrate the validity of the principles on which its operation was based.

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I. INTRODUCTION

It has been established for some time (Barber, 1911; Chambers, 1922) that it is feasible to penetrate and withdraw micropipettes, probes, and other instruments from some kinds of living cells. With the appearance of papers by Hodgkin and Huxley (1939), Curtis and Cole (1940), interest began to manifest itself in utilizing some sort of intracellular electrode to record active membrane potentials in electrically active cells. It was, however, not until Gerard and his associates (Graham and Gerard, 1946; Ling and Gerard, 1949) demonstrated the usefulness of the ultramicropipette to record intracellular potentials in muscle fibers that it became apparent that a technique was available to record directly from the interior of a wide variety of cells.

These investigators observed that when the tip diameter of the pipette was of the order of 0.5 micron or less, penetration of intact cell membranes was possible with little evidence of cell injury - at least for a period long enough to obtain considerable information regarding the static and dynamic states of the membrane potential. This observation has been confirmed by a number of investigators in many different types of electrically excitable cells (Nastuk and Hodgkin, 1950; Woodbury, Woodbury and Hecht, 1950; Brock, Coombs and Eccles, 1951; Woodbury and Patten, 1952; Eccles, 1952; Hartline, MacNichol and Wagner, 1953). The pipette is usually filled with a salt solution. The choice of electrolytes and concentrations has varied with different investigators. A popular choice of late has been potassium chloride in 2 or 3 molar concentration.

Essentially the pipette may be considered a salt bridge from the interior of the cell to some external nonpolarizable metal electrode connected to the amplifier. It suffers from a serious defect, that is its high impedance. Pipettes having tip diameters of less than 0.5 micron have resistances of from 10 to 50 megohms when filled with 3 M KCl and hundreds of megohms when filled with isotonic Ringer's solution. This high input impedance introduces objectionable features if the measured signal is led directly from the pipette to the input stage of a conventional physiological amplifier. One of these features arises if grid current flows through this impedance. An appreciable IR drop can be present and would have to be considered in any d. c. measurements. Since grid currents drawn by ordinary vacuum tubes used in amplifiers are usually of the order of 10^{-10} amperes, across a 100 megohm electrode it would develop a 100 millivolt d. c. signal. Nor is the impedance of micropipettes constant in use, for with plugging or breaking of the tip, as frequently occurs, large shifts in impedance are seen. Clearly it is desirable to make the IR drop so small as to not require consideration.

There is an additional reason for achieving a low grid current. It has been our experience as well as that of others (Tasaki, 1953; MacNichol, Wagner, Hartline, 1953) that currents of the order of 10^{-10} amperes are adequate to alter the electrical excitability of nervous tissue. We have, for this reason, felt it desirable to specify a grid current of less than 10^{-12} amperes. More experience and more sensitive tests may enable a more careful estimate of a safe value to be made.

An even more serious consequence of the high impedance electrode is the effect on the response time. If the effective input capacitance of the electrode and amplifier were $10 \mu\text{f.}$, a not unreasonable figure, and if the electrode had a resistance of 10^8 ohms the input circuit would have a time constant of 10^{-8} seconds. This is entirely too slow for following the excitatory events in neurons. Thus it is evident that special input circuits must be employed to fulfill two important conditions, very low grid current, and very small effective input capacitance.

Ordinary radio receiving tubes have grid currents of from 10^{-8} to 10^{-10} amperes when operated under normal conditions. Careful selection and aging of some types such as the 6AK5 and 5879 have yielded a few units having grid currents of 10^{-11} amperes or less. Electrometer

tubes are available having grid currents as small as 10^{-10} amperes; but as ordinarily used, these tubes do not permit achievement of a small input capacitance and consequent short response time. It will be shown later that they can be used in multistage feedback-amplifiers in such a way that very small grid current and short response time can be achieved simultaneously.

II. THE USE OF NEGATIVE FEEDBACK

As far as the authors are aware all the input stages currently in use for recording action potentials from micropipette electrodes make use of negative feedback to increase the input impedance of the amplifier and to stabilize its gain. A review of the elementary feedback theorems will explain why feedback is effective (Williams, 1949; Eode, 1945).

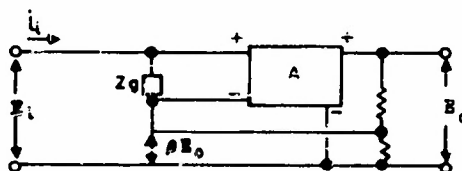


Figure 1. - Schematic of a simple feedback amplifier.

Consider an amplifier having a gain A between its input and its output terminals (fig.1)*. It has an input impedance Z_g and is so connected that a fraction β of the output signal voltage E_o is fed back to the input circuit and subtracts from the input signal E_i .

The signal at the grid of the amplifier $E_g = E_i - \beta E_o$.

Since the amplifier has gain A , $E_o = A E_g$.

Substituting for E_o , $E_g = E_i - A \beta E_g$ and

$$\frac{E_i}{E_g} = 1 + A \beta.$$

Let the amplifier have an input impedance $Z_i = \frac{E_i}{I_i}$. The source of this input impedance is the grid circuit impedance Z_g of the amplifier which is the only path through which the input current I_i flows so that

$$I_i = \frac{E_g}{Z_g}.$$

Substituting for I_i ,

$$Z_i = \frac{E_i}{E_g} Z_g \text{ and for } \frac{E_i}{E_g} \text{ we have } Z_i = Z_g (1 + A \beta).$$

*In this analysis A and β are positive quantities and β is subtracted from E_i . In some analyses β is given as a negative quantity which is added to E_i . The two conventions are entirely equivalent and differ only that in one case the term $(1 + A \beta)$ appears in the final expressions and in the other case $(1 - A \beta)$ appears.

Thus the introduction of feedback gives an input impedance which is $(1 + A\beta)$ times as large as the grid circuit impedance. In the frequency region in which the phase shift of the amplifier itself is negligible the grid circuit impedance appears as a dynamic grid circuit resistance r_g in parallel with a capacitive reactance C_g and both of these components are increased by a factor $(1 + A\beta)$ so that the effective input resistance $R_i = r_g (1 + A\beta)$ and capacitance $C_i = C_g / (1 + A\beta)$. The decrease in effective capacitance of the amplifier results in a shortening of the input time constant. If the source of the input signal has a resistance $R \ll R_i$, the input time constant will be $RC_i = RC_g / (1 + A\beta)$ instead of RC_g and the frequency passband will be increased proportionately. The frequency at which the output voltage drops to 0.707 of its maximum value (half-power point), will shift from

$$\frac{1}{2\pi RC_g} \text{ to } \frac{1 + A\beta}{2\pi RC_g}.$$

In addition there is an improvement in the stability of the effective voltage gain γ of the amplifier and feedback circuit with changes in factors that affect the gain A of the amplifier alone such as tube-aging, changes in the supply voltages, etc.* This can be shown as follows: $E_g = E_o/A$ and $E_o/A = E_i - \beta E_o$

so that the gain $\gamma = \frac{E_o}{E_i} = \frac{A}{1 + A\beta}.$

Differentiating, $\frac{d\gamma}{dA} = \frac{1}{(1 + A\beta)^2}$ and expressing in terms of fractional changes in A

$$\frac{d\gamma}{\gamma} = \frac{1}{1 + A\beta} \times \frac{dA}{A}.$$

Therefore changes in A bring about much smaller changes in net gain when feedback is present; the fractional change in gain being reduced by the factor

$$\frac{1}{1 + A\beta}.$$

III. THE CATHODE FOLLOWER

The first successful and still most widely used device for coupling a micropipette electrode to an amplifier is the cathode follower (fig. 2). It amounts to a single-stage amplifier in which the entire output signal is subtracted from the input, that is, $\beta = 1$. The improvement that can be obtained from such a device is indicated by the following typical example.

A high performance pentode such as the 6AK5 can provide a voltage gain of at least 100 so that $1 + A\beta = 101$ and the effective gain

$$\gamma = \frac{100}{101}.$$

The effect of the input capacitance comprising the grid to cathode and grid to screen capacitance is reduced by a factor of 101 as previously shown. A further improvement is obtained

*In accordance with a common usage the gain A of the amplifier without feedback will be referred to as "the open-loop gain". The effective gain when feedback is operative will be termed "the closed-loop gain". In this report gain will be expressed as the ratio of the output voltage to the input voltage. Logarithmic units will not be used.

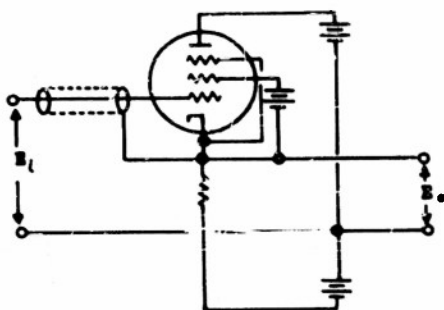


Figure 2a. - Cathode follower typical circuit.

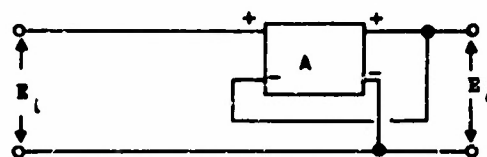


Figure 2b. - Equivalent schematic of figure 2a.

by enclosing as much of the lead to the electrode as possible in a shield connected to the cathode of the cathode follower where it is in parallel with the grid circuit capacitance. For example, suppose the input lead has a capacitance of $5 \mu\text{f.}$ to ground. Enclosing the lead in a shielded cable results in removing the capacitance to ground and substituting $20 \mu\text{f.}$ or so of cable capacitance to the cathode. As far as the input time constant is concerned this is equivalent to a capacitance of $20/(1 + A\beta) \mu\text{f.}$ or if the circuit of the previous example were used $0.2 \mu\text{f.}$ instead of $5 \mu\text{f.}$ so that the input time constant would have been decreased by a factor of 25.*

Unfortunately the cathode follower is no solution to the grid current problem. If we are to achieve a large value of open-loop gain A in order to take advantage of feedback in reducing input capacitance we must use a vacuum tube under conditions in which the amplification factor and transconductance are large. These are achieved only when a large space current flows in the tube. A large space current produces a large grid current because electrode potentials in excess of the ionization potential of the residual gas are required to produce this current and because the rate of production of gas ions is directly proportional to space current.

It is possible to operate a cathode follower at "the crossover point" and obtain a very small d. c. component of grid current. Crossover occurs at a bias of about -0.75 v. to -1.3 v. depending upon cathode temperature. It is a condition in which just enough electrons emitted from the cathode have sufficient thermal energy to reach the grid and produce a current equal and opposite to the sum of the currents produced by positive ion collection, and photoelectric and thermal electron emission. The neighborhood of the crossover point is a very unsuitable operating region. In the first place, the grid current changes markedly with slight changes in heater temperature and with slight shifts in the operating point. Secondly, the change in grid current with change in signal voltage (the dynamic resistance) of the grid is very much smaller than when the grid is biased more negatively. Most tubes have a "plateau" of grid current at biases more negative than about -1.2 to -2.0 volts in which grid current is practically independent of grid potential over a very wide range. This signifies a high dynamic grid resistance and the choice of the operating point is not critical. In some cases it may be possible to buck out a large fraction of the plateau grid current by means of a steady potential applied to a fixed resistance. However, such a procedure introduces a critical adjustment and the input resistance is decreased by the added resistor.

IV. MULTISTAGE AMPLIFIERS

A better solution to the problem consists of using a tube having a very small plateau value of grid current and making up for the inherent lack of gain by means of one or more additional stages of amplification inside the feedback loop. Thus an electrometer tube can

*Dr. K. Frank of the National Institutes of Health has made elegant use of this principle by shielding his microelectrodes to within 100 micra or so of the tip by means of a varnished platinum coating.

be operated as an "input sensing" device for an amplifier having a large open-loop gain determined by subsequent stages. As shown earlier the fractional change in gain is reduced by a factor

$$\frac{1}{1 + A\beta}$$

If $A\beta$ is much larger than unity, changes in A due to nonlinearity become unimportant.

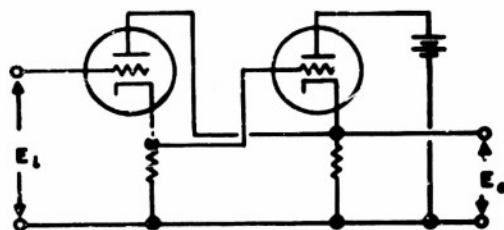


FIGURE 3

Electrometer triode follower
(Krakauer 1953).

Various circuit configurations for using an electrometer tube have been suggested. The cathode-coupled circuit has been used very successfully by Krakauer (1953). This configuration is shown in figure 3. The plate supply of the electrometer tube is "floated" on the cathode of the second tube in such a way that the second stage operates without feedback. Overall feedback for both stages is provided by the voltage drop across the cathode resistor of the first stage. When a tube having a large amplification factor and large transconductance is used in the second stage this circuit is capable of excellent performance. Krakauer's paper describes an amplifier in which an effective input capacitance of $0.1 \mu\text{f}$, and a grid current of 2×10^{-14} amperes were achieved. This circuit does not however lend itself to the use of the negative-capacitance scheme to be described later.

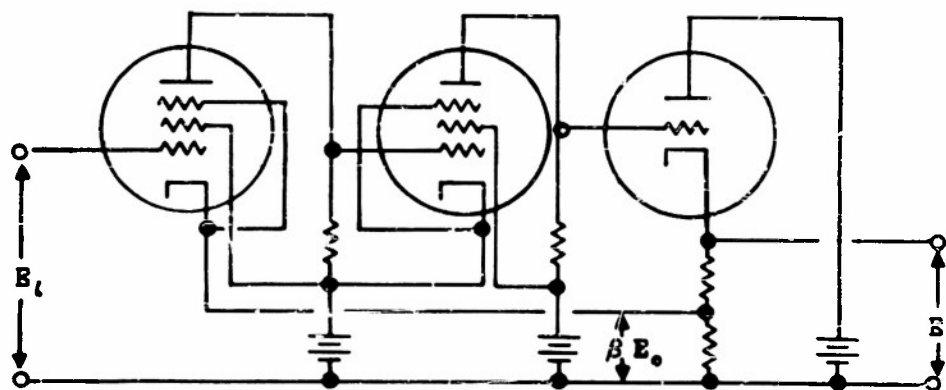


Figure 4. - Typical configuration of multistage feedback amplifiers.

Another possible configuration is shown in figure 4. This is the familiar inverse-feedback pair widely used in audio-frequency systems. We have selected this arrangement as most nearly fulfilling the requirements. The electrometer tube is directly coupled to a pentode operated under conditions in which a large voltage gain is produced. To obtain a large gain the plate load resistor of the pentode must be large. A cathode follower is therefore used between the pentode stage and the low-resistance feedback network. If the amplifier had an open-loop gain A of 100 and a feedback factor β of 1, the input time constant would be reduced by a factor of 101 as in the previous example of the cathode follower. The grid current would, of course, be much less due to the use of the electrometer tube. As in the cathode follower much of the

effective capacitance of the input lead could be removed by enclosing this lead in a shield connected to the cathode of the electrometer tube. However, this procedure does not remove the effects of the capacitance of the electrode itself.

V. NEGATIVE IMPEDANCE

The micropipette electrodes have a capacitance of about $1 \mu\text{f}$. per millimeter of penetration of the tip into solution (Nastuk and Hodgkin, 1950). If the electrode were immersed 5 mm. and had a resistance of 100 megohms the time constant would be 0.5 milliseconds. The time required to reach 90 percent of full output would be about 3 time constants or 1.5 milliseconds. The spike potentials produced by excitable tissues are usually of shorter duration than this so that it would not be possible to measure the magnitudes of these potentials with such a slow recording system. It would be entirely out of the question to measure the time course of such potential changes. Therefore, it is necessary to reduce the effective capacitance of the electrode itself, as well as the capacitance of the amplifier and input lead. There are at least two ways of compensating for the time constant associated with the electrode. One is to insert an equalizing network somewhere in the amplifier system (P. R. Bell, 1949B). Such an equalizer would have a change in voltage attenuation and a phase shift with change in frequency that are so adjusted that the product of the voltage attenuations and the sum of the phase shifts introduced by the electrode and network in series are constant. Thus the network would undo the harm that was done to the signal by the electrode. This method has been used extensively and has found favor especially in nuclear physics instrumentation. It is quite satisfactory when used with proportional particle counters and ionization chambers since both R and C are fixed. For electrophysiological work this method has the serious disadvantage that it does not reduce the current required to charge the input capacitance. Usually the capacitance of the input circuit remains moderately well fixed during an experiment (provided the depth of insertion of the electrode does not change greatly). However, the input resistance may vary over a wide range due to plugging of the electrode tip by tissue debris and due to changes in the resistance of the tissue itself. Furthermore the tissue resistance is usually a function of the current flowing through it. Although, as shown above, the d. c. grid current can be made very small and the variational resistance of the grid circuit very high, the current required to charge the input capacitance cannot be neglected. This current is proportional to the rate of change of the input signal and amounts to 10^{-12} ampere per micromicrofarad per millivolt per millisecond. This would amount to 10^{-8} amperes with an input capacitance of $10 \mu\text{f}$. and a rate of rise of 100 mv/msec. (a not unusual situation). A current of this magnitude could cause serious distortion in the recording of the potential changes produced in a single cell and may cause changes in the state of the cell itself.

The second way of correcting for the input time constant involves making the effective input capacitance arbitrarily small. This also decreases the current drawn by the input circuit during rapid changes in signal voltage so that the objection to the first method does not apply.

A method of making the effective capacitance of a circuit arbitrarily small consists of adding a negative capacitance of a magnitude nearly equal to the unwanted capacitance. In an amplifier this is accomplished by feeding back a portion of the output signal in such a way that a current is obtained which is just equal and opposite to the charging current in the unwanted capacitance. This principle is not new and was first employed in the "neutrodyne" circuit widely used in radio receivers during the 1920s.

The circuit configuration to be described is essentially that developed by P.R. Bell, (1949A) and is shown schematically in figure 5. Basically similar circuits have been used by Solms, Nastuk, and Alexander (1953) and by Woodbury (1953).

In principle it is possible to obtain a negative resistance, capacitance, inductance, or a combination of these elements. The following simplified analysis shows how a generalized negative impedance can be obtained.

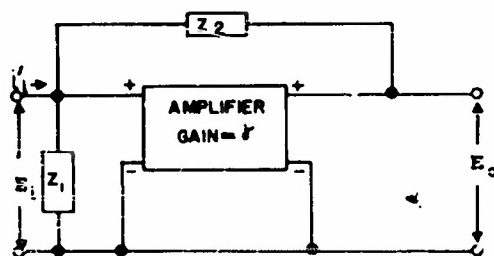


Figure 5. - Schematic of a negative impedance circuit.

In an amplifier having a variational input impedance Z_1 and a gain γ , an impedance Z_2 is connected between the output and the input terminal. A signal source impresses a potential E_i upon the input terminal which produces an output potential $E_o = \gamma E_i$. The signal source must supply a current:

$$I_i = \frac{E_i}{Z_1} + \frac{E_i - \gamma E_i}{Z_2} = E_i \left[\frac{1}{Z_1} + \frac{1 - \gamma}{Z_2} \right] = E_i \left[\frac{1}{Z_1} - \frac{\gamma - 1}{Z_2} \right].$$

The effective input admittance Y_i of the whole circuit is defined as the ratio of input current to input potential or reciprocal of the effective input impedance Z_i , that is

$$Y_i = \frac{1}{Z_i} = \frac{I_i}{E_i} = \frac{1}{Z_1} - \frac{\gamma - 1}{Z_2}.$$

Thus it appears that Z_i is made up of the parallel combination of Z_1 and a negative impedance $\frac{\gamma - 1}{Z_2}$. It is desired to make the amplifier follow the input signal without drawing any current

(other than d. c. grid current) from the signal source, that is to make $I_i = 0$. Since E_i is in general not zero Y_i must become zero and Z_i must become infinite. Putting $Y_i = 0$ gives

$0 = \frac{1}{Z_1} - \frac{\gamma - 1}{Z_2}$ so that $\frac{1}{Z_1} = \frac{\gamma - 1}{Z_2}$ and $Z_2 = Z_1 (\gamma - 1)$. Thus to make the input impedance of

the amplifier infinite Z_2 must be the same kind of an impedance as Z_1 differing from it only in magnitude by a constant factor $(\gamma - 1)$. If Z_1 is a resistance or an inductance, Z_2 is a resistance or an inductance $(\gamma - 1)$ times as large. If Z_1 is a capacitance, Z_2 must be a capacitance $\frac{1}{\gamma - 1}$ times as large. If Z_1 is a parallel combination R_1, C_1 then Z_2 must be a parallel combination $(\gamma - 1) R_1, C_1/(\gamma - 1)$.

It is evident that $\frac{\gamma - 1}{Z_2}$ must not be allowed to become larger than $\frac{1}{Z_1}$ because if it

were, current would flow in such a direction as to increase the input signal so that the system would have a gain greater than γ (a principle made use of in regenerative amplifiers but producing conditions too unstable for the present application). Indeed if our signal

source had an impedance Z and $\frac{\gamma - 1}{Z_2} > \frac{1}{Z} + \frac{1}{Z_1}$, the gain of the system would become infinite and we would have a trigger circuit. In practice it is best to keep $\frac{\gamma - 1}{Z_2}$ always slightly less than $\frac{1}{Z_1}$ so that regenerative amplification cannot take place. The input impedance will then have a finite value which can be made arbitrarily large by suitable design.

It is evident then an approach to infinite impedance places a premium on the stability of γ since the magnitude of the negative impedance $\frac{Z_2}{\gamma-1}$ depends critically upon γ . Hence it is desirable to use negative feedback with $A\beta \gg 1$ as explained in the preceeding section.

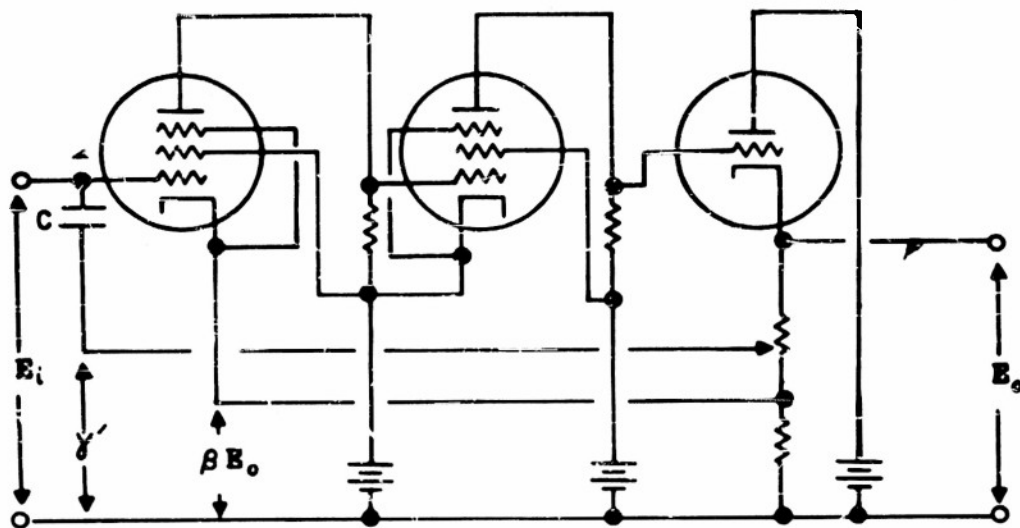


Figure 6. - Modification of the amplifier in figure 4 to provide capacitance feedback.

In an electrometer tube amplifier the resistive component of the input impedance is already many thousands of megohms and the use of a large amount of negative feedback makes it still greater. Only the capacitative reactance needs to be compensated which can be accomplished by feeding back through a small variable capacitor C. A more convenient way for our purposes since it lends itself more readily to remote control is to feedback through a variable resistive attenuator and fixed capacitor. Thus the effective value of γ is changed while C remains constant (figure 6). It has been found particularly convenient to use the capacitance of the cable between the electrode and the input circuit as the balancing capacitor.* It is connected to a potentiometer that forms a portion of the negative feedback (β) network of the preamplifier as shown in the simplified circuit diagram (fig. 6). Except for the connection to the cable shield the amplifier is identical to the one shown in figure 4.

VI. PRACTICAL APPLICATION TO AN AMPLIFIER

In our preliminary considerations before undertaking the design of a preamplifier we felt our experimental needs would be satisfied if the following requirements could be obtained:

- (a) A stable voltage gain of two or three.
- (b) Grid current of less than 10^{-12} amperes.
- (c) Input time constant of 100 microseconds when used with an electrode having a resistance of 100 megohms and a capacitance of 5 μmf .
- (d) An input dynamic range of at least ± 200 millivolts.
- (e) Means for bucking out steady input signals (zero suppression).

*This can be accomplished satisfactorily only when a cable is used that has a capacitance that is nearly independent of the degree of flexion of the cable. Flexible coaxial cables filled with semi-solid dielectric, especially those using tetrafluoroethylene (teflon) fulfill this requirement.

- (f) Means for measurement of electrode resistance and input time constant with the electrode in place.
- (g) It was desirable that the unit be physically small and remotely operated in order that it could be attached mechanically to a micromanipulator.

It can be seen, particularly with respect to the input time constant, that we did not place as severe a requirement as might have been necessary had we been working with fast mammalian nerve fibers. In our preparations, *Limulus* and frog, events were slow enough that this seemed adequate at the time.

Before considering the need to go to electrometer tubes (which have some disadvantages such as the very large microphonic effects caused by the use of a filamentary cathode) an examination was made of some standard receiving type tubes that could be operated under electrometer conditions. The 5879 pentode seemed acceptable when operated under the conditions shown in table 1.

Table 1

Heater voltage	4.5 volts
Plate voltage	+ 20.0 volts
Control grid voltage	- 1.5 volts
Screen grid voltage	+ 21.0 volts
Plate current	2 microamperes
Transconductance	8 micromhos (approximately)

Under these conditions half the tubes (R.C.A.) tested had grid currents of less than 10^{-12} amperes which were constant over a range of grid voltages of from -1 to -3 volts. Life testing of 4 tubes for 200-hour periods showed negligible change in operating characteristics after the first 24 hours of operation. With the choice of this tube type for the "input sensing" device an amplifier design was established (fig. 7) upon the following considerations, a first stage voltage gain of approximately 4 was obtained by using a plate load resistor of 560 k ohms. The interstage capacitance was estimated to be about 10 μmf . which would yield an output time constant of 5 μ seconds. This was made the limiting time constant of the system, (i.e., a time constant that is much longer than all the others).

It was found convenient to elevate the cathode of the second stage to the +22.5 v. tap on the supply battery in order to allow direct coupling to the first stage. The total positive supply potential was limited to +90 v. This permitted the plate of the second stage to operate at +60 v. Although the plate to cathode potential was only 37.5 v. with a screen potential of 67.5 v., the second stage tube (5879) still had a transconductance of 1000 micromhos at 1 ma. of plate current yielding a voltage gain of about 27. Since it was desirable for the output signal to vary about ground potential the level of the output signal from the plate of the second stage was decreased by use of a voltage divider to the 90 v. negative supply. This attenuated the signal by a factor of 0.6 but the method appeared to be more satisfactory than the alternatives available. For instance, a coupling battery was out of the question due to the space required and the large capacitance to ground. A neon lamp could not have been used because these devices are too noisy to use at signal levels in the millivolt range.

Both sections of a 12A17 operated in parallel were used as an output cathode follower with a quiescent current of 2 ma., drawn from the 90 v. negative supply, positive or negative output signals of about 5 v. peak were permitted. Adjustment of the output level was accomplished by varying the plate supply potential of the first stage of the amplifier.

Wire wound resistors in the feedback network were necessary since stability and low particle noise were of extreme importance. Noise generated in the bottom resistor of the feedback network is not reduced by the feedback so that it is important that this resistor be of the highest quality. Coupling resistors were of the ordinary composition type and undoubtedly did contribute some noise. Carbon film resistors would be preferable.

A 1.5 v. dry cell was used to bias the first stage to permit operation of the input grid at ground potential, an arrangement that minimizes leakage currents. To reduce leakage even further, it was found desirable to use a ceramic tube socket coated with a silicone material (a teflon socket would be better) and to coat the bottom of the input tube itself with this material ("Dessicote" made by Beckmann Instrument Company, California, has been found to be especially satisfactory). It is important that the input cable have the highest possible leakage resistance. "Teflon" cable* and connectors (resistivity 10^{12} ohm cm.) is probably best in this respect with polyethylene nearly as satisfactory from the leakage standpoint (10^{12} ohm cm.). However, polyethylene cable has an unpleasant "microphonic" effect due to charge separation when the cable is flexed. This effect is present to a much smaller degree in teflon cable.

We used the 400 series Jones plugs, and rotary switches in which several contacts were operated in parallel, to secure very low and constant resistance in connectors and battery switching circuits.

The auxilliary circuits that were used for calibration and zero suppression are shown in figure 8. The lower portion of the diagram represents the bucking circuit which consisted of a battery and calibrated potentiometer used to insert a steady potential between the indifferent electrode and the grounded side of the amplifier input. The potential was used to measure and to oppose large steady potentials arising within the preparation so that small potential changes could be measured at high amplifier gain.

The upper portion of the diagram represents a source of calibration potential controlled by a hand key. The output voltage could be applied either in series with the bucking voltage ("calibrate" position) or through an individually shielded cable to a 10^{10} ohm glass-enclosed resistor (R_1 in figure 7) the other end of which was permanently connected to the input grid, ("test" position). This circuit permitted the measurement of grid current, electrode resistance, and input time constant. It was also found useful for electrically polarizing tissue which was being investigated directly through the micropipette.

Grid current was measured by first balancing the amplifier for zero output with the input short-circuited by plugging the ground lead into the electrode holder; then removing the short circuit, the potential was determined that must be applied through R_1 to restore the output to zero. Then

$$I_g = \frac{E}{R_1}.$$

To determine the electrode resistance R two measurements were necessary. First the electrode was inserted into solution and the reference electrode grounded. An arbitrary potential E_1 was applied to R_1 resulting in a deflection of the oscilloscope connected to the amplifier equivalent to a voltage E_g at the grid. Next, R_1 was grounded and a potential E_2 was applied to the reference electrode sufficient to produce the same deflection of the oscilloscope as in the previous case. The resistance R between the grid and the reference electrode was found directly since

$$E_g = E_1 \frac{R_1}{R_1 + R} = E_2 \frac{R}{R_1 + R}, \text{ so that } R = \frac{E_1}{E_2} R_1.$$

*Amphenol type 21-382 or military specification RG 126-U cables are satisfactory.

The measurement of the input time constant can in theory be accomplished by applying a step change in voltage to the 10^{10} ohm resistor and observing the rate at which the output voltage changes. If a potential E is applied, the voltage at the grid will be.

$$E_g = E \frac{R}{R+R_1} (1 - e^{-t/R'C}).$$

Where

$$R' = \frac{R \cdot R_1}{R+R_1}.$$

The effective time constant of the input circuit will be the time at which $t = R'C$ when the output reaches 63 percent of its final value.

This method of measurement supposes that R_1 is a pure resistance having no capacitance associated with it, a condition not realizable in practice. Actually there is a distributed capacitance along the length of the resistor and a distributed capacitance to ground. The effect of the lengthwise distributed capacitance is to present a lower impedance to the high-frequency components in the signal than to the low-frequency components. This results in a signal that has too fast a rise, making the response of the input circuit appear better than it actually is. On the other hand, the capacitance to ground attenuates the high-frequency components in the signal. The ratio of distributed capacitance to capacitance-to-ground can be altered by passing the resistor through a hole in a shield barrier that can be moved back and forth along the resistor. When the shield is nearest the grid end, the capacitance-to-ground predominates; when it is at the input end, the lengthwise distributed capacitance predominates. The best location unfortunately depends upon the resistance and capacitance in the rest of the circuit; but that is what we are trying to adjust! To date no good solution has presented itself.

Some workers have estimated the circuit time constant by applying a square wave in series with the reference electrode. This procedure may give an erroneous result because the electrode capacitance is then in series with the signal source. When the preparation is active the capacitance is in parallel with the input circuit giving a slower response. Thus the measurement makes the response of the circuit appear more rapid than it actually is.

VII. RESULTS

During a year of routine operation the grid current in the first stage remained stable in the region of 3×10^{-13} to 5×10^{-13} amperes. Small fluctuations occurred. Apparently this was due to changes in surface leakage resistance with changes in atmospheric humidity.

A tendency toward oscillation at a frequency of several hundred kc. appeared and was cured by compensation of the feedback network with mica capacitors.

Response of the circuit to square wave input through simulated electrodes is shown in figure 9. Record (a) shows a 5 kc. square wave input signal applied directly to the oscilloscope. Records (b), (c), and (d) were taken with a dummy electrode consisting of a 200 megohm carbon film resistor passed through a hole in a grounded partition. They show the effects of no compensation, correct compensation, and over compensation for the stray input capacitance respectively. Record (e) shows the effect of moving the shield partition from the input end to the grid end of the resistor without changing the compensation adjustment used when record (c) was obtained. As mentioned earlier this changes the ratio of lengthwise distributed-capacitance to capacitance-to-ground. It is evident from the record that these capacitances associated with large resistors cannot be ignored.

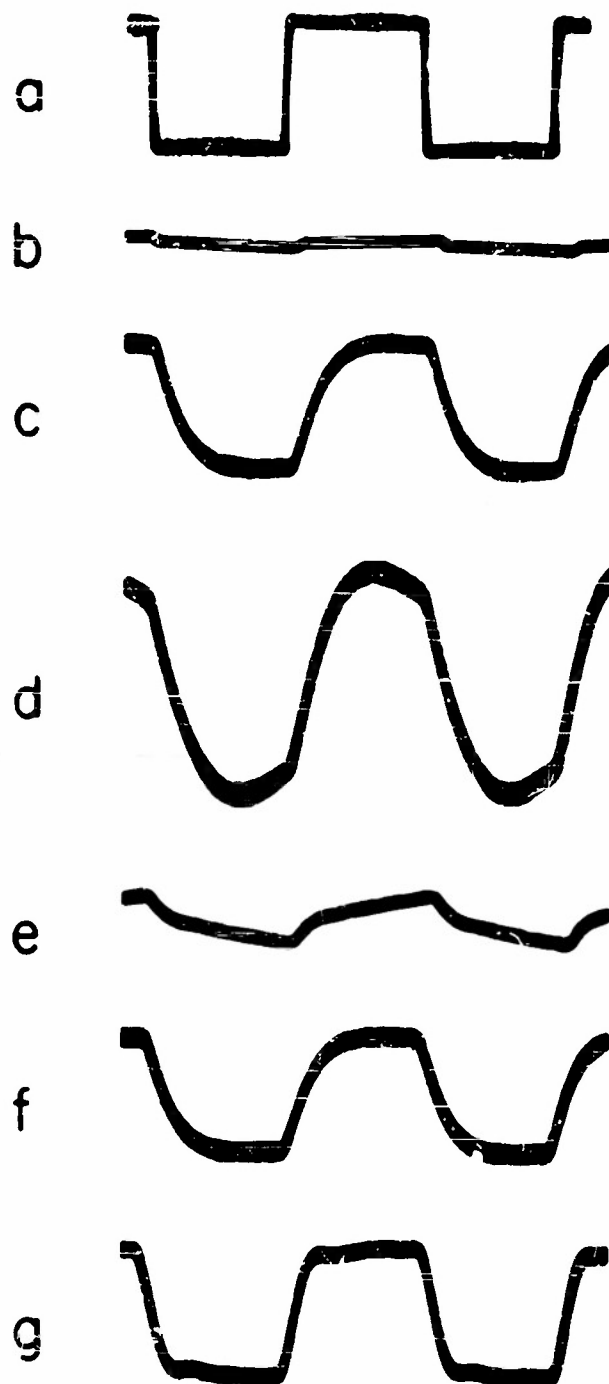


Figure 9. - Oscillograms demonstrating response characteristics of the preamplifier to a square-wave input. See text for detail discussion of individual traces.

Record (f) shows the effect of placing a 10 $\mu\text{mf.}$ mica capacitor from grid to ground and then adjusting the negative capacitance compensation for best response. This record is practically indistinguishable from record (c) in which correct compensation was obtained without any added capacitance. Thus the circuit is able to correct for 10 $\mu\text{mf.}$ external capacitance. The improvement in response time is really quite striking. The time constant of 200 megohms and 10 $\mu\text{mf.}$ is 2,000 microseconds. The effective time constant obtained from the record is only 20 $\mu\text{sec.}$, a factor of 100 improvement. Record (g) shows the effect of a 10 megohm input resistor with 10 $\mu\text{mf.}$ capacitance from grid to ground. The effective time constant in this case is still in excess of 10 microseconds. It is evident that with source resistances of the order of 10 megohms the reduction in effective time constant is not as great as with resistances in excess of 100 megohms. This effect is due to the limited band width of the amplifier. In the simplified analysis given in this report an ideal lag-less amplifier was assumed. Actually there is a time delay due to the resistances and stray capacitances associated with the coupling circuits. This means that the negative feedback at a given frequency is not exactly 180° out of phase with the input signal nor is the positive feedback exactly in phase with the signal. At frequencies where the phase shift is 90° the feedback does no good whatever. If the phase shift exceeds 90° the sense of the feedback becomes reversed. In the amplifier under discussion the phase shift is limited to 90° by having a single time constant much greater than the others. Since a single RC circuit cannot have a phase shift greater than 90° it is only necessary that this circuit produce sufficient attenuation at frequencies at which the other coupling circuits can have appreciable phase shift. If the gain is less than unity at these frequencies the circuit cannot oscillate.

As mentioned previously the coupling circuit between the first two stages furnished the controlling phase shift. The time constant was estimated to be 5 microseconds. The frequency at which the output voltage would be reduced to 0.707 its low-frequency value would be

$$f = \frac{1}{2\pi T} = 32 \text{ KC.}$$

Figure 10 shows that this estimate is somewhat optimistic. The measured

frequency was about 19 KC corresponding to a time constant of about 8 $\mu\text{sec.}$ The curves corresponding to the filled circles show the amplitude and phase characteristics of the amplifier with the negative feedback loop open. Closing the feedback loop* resulted in a marked improvement in the high frequency response as shown by the curves corresponding to the open circles. In this case the gain began to fall off and phase shift became appreciable at about 100 kc. If a still smaller effective time constant were required it would be necessary to design an amplifier having better high-frequency response with the feedback loop open. This could be accomplished by decreasing the value of the plate resistor of the first stage and making up for the loss in gain by the use of a wide-band amplifier having several stages inside the feedback loop. However, the present amplifier was adequate for the purposes for which it was being employed.

VIII. NOISE FIGURE

The preamplifier was designed to work at signal input levels in excess of 1 mv. but since some applications may require operation with much smaller signals, tests were made to determine the noise contributed by the tubes and circuit and to compare it with the noise produced in several values of input resistance. An oscillator (Hewlett-Packard 200c) was connected to the preamplifier through a calibrated attenuator (General Radio Microvolter). Measurements were made with several values of resistance inserted in series between the attenuator and the preamplifier input terminal. The preamplifier was connected to a d. c. amplifier and oscilloscope having a response which was constant from zero frequency to several

*It should be noted that the curves shown in figure 10 were taken under test conditions in which $\beta=1/2$ rather than $1/3$. With $\beta = 1/3$ the feedback would not improve the high-frequency response as much as the curves indicate.

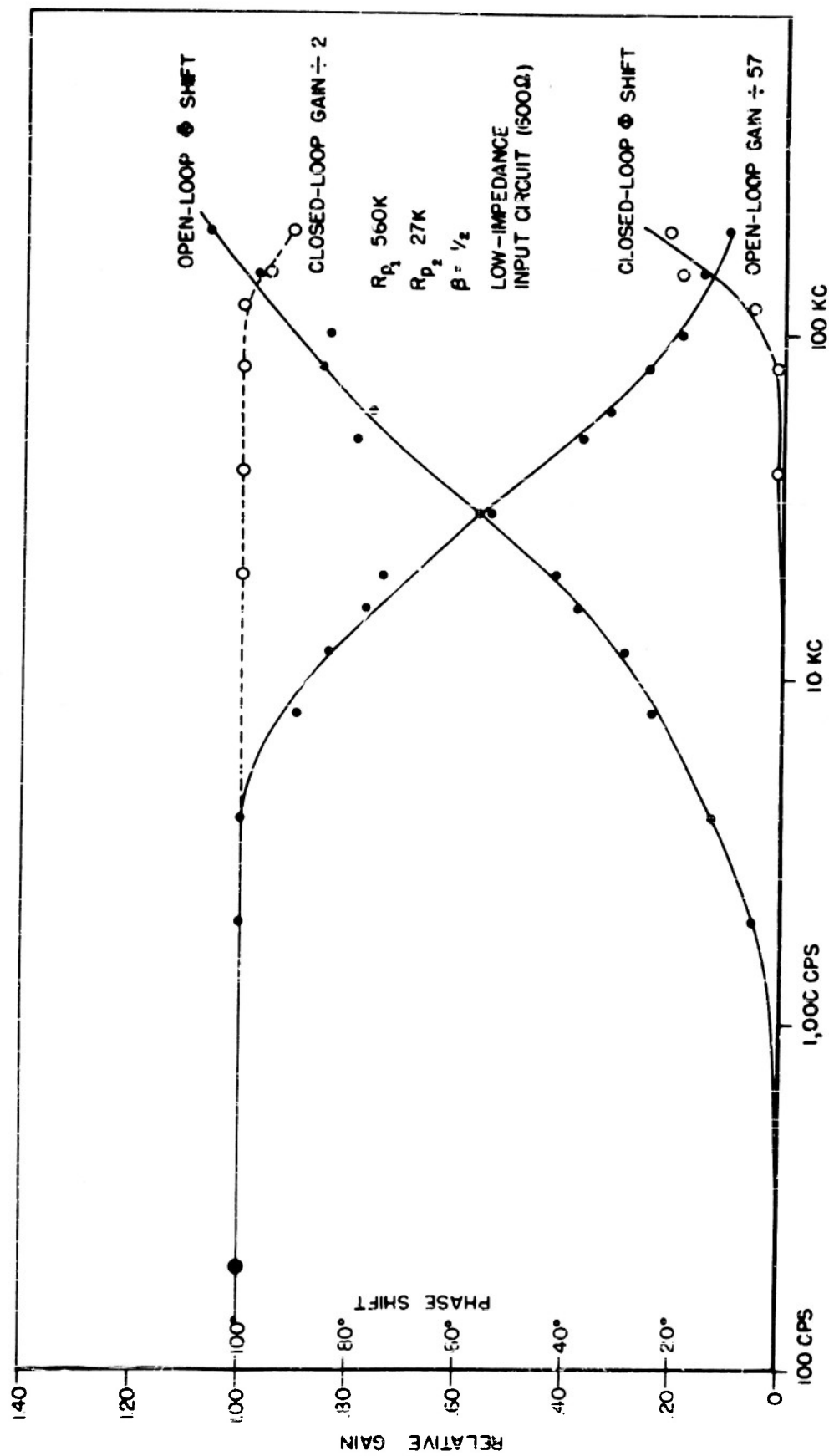


Figure 10. - Frequency and phase characteristics of amplifier.

kilocycles and which dropped smoothly to half voltage at 10 kc. The negative capacitance control was adjusted for each value of input resistance so that the half-voltage point of the overall system was maintained at 10 kc. A 1 kc. signal from the oscillator was adjusted until the signal was judged equal to noise on the persistent (P7) screen of the oscilloscope. Because of the inaccuracy of the measuring technique several readings were taken and averaged as shown in table 2.

Table 2

Input resistance	Signal judged equal to noise	Theoretical noise
600 ohms	5.5	Average 4.9 μ v. Negligible
	5.0	
	4.5	
	4.0	
	4.6	
1 megohm (Shallcross Wire Wound)	11	Average 12.8 μ v. 13 μ v.
	14	
	12	
	13	
	14	
	13	
10 megohms (I.R.C. Carbon Film type D.C.H.)	38	Average 37.6 μ v. 40 μ v.
	36	
	39	
	39	
	36	

The theoretical noise in a pure resistance at 300° K. is given by $\bar{E}^2 = 1.64 \times 10^{-20} R \Delta f$. Where \bar{E} is the r. m. s. noise voltage, R is the resistance and Δf is the frequency band over which the measurement is taken. The noise that should have been generated in each input resistance is also shown in the table. It is evident that the measured values of noise are too low compared with the theoretical values especially when the 4.9 μ v. of tube and circuit noise are subtracted from the experimental figures. The noise values for 1 meg. and 10 meg. are in nearly the correct ratio to one another

$$\left(\sqrt{\frac{1}{10}} \right)$$

and the scatter in the measurements is quite small. Apparently there is a systematic error in the data. Probably the wrong criterion was consistently used in judging the signal equal to noise and the actual effective bandwidth was probably less than 10 kc. Since the high frequency cutoff of the measuring system was not perfectly sharp there was some attenuation below 10 kc. and some output above 10 kc. the net contribution of these two opposing effects should be taken into account in an accurate measurement. It is probable that the half-power (0.707 voltage) point should have been used to specify the bandwidth. (This would have made the bandwidth somewhat narrower.)

Even if it is assumed that the measurements were in error by a factor of two, the important point, however, is that the effective noise in the input circuit was not markedly increased over the theoretical value by use of a negative capacitance. Furthermore, it appears that the input tube and circuit alone were not excessively noisy.

As an additional check the tube and circuit noise were measured by an independent method. A variable resistance was connected across the input circuit and the value of resistance

necessary to produce a noise output double that of the short-circuited amplifier was determined. Ten measurements were made as shown in table 3. The average value of resistance producing noise equivalent to that produced by the preamplifier was found to be 137 k. This resistance should theoretically produce a noise of $4.75 \mu v.$ in a 10 kc. bandwidth; a figure in good agreement with the $4.9 \mu v.$ determined by the direct method.

Table 3

Measurement of tube and circuit noise by resistance substitution

R	
220 k	} Average 137 k Calculated Noise in 137 k = $4.75 \mu v.$
150 k	
100 k	
180 k	
48 k	
100 k	
150 k	
140 k	
140 k	

BIBLIOGRAPHY

- Barber, M.A. A technique for the inoculation of bacteria and other substances into living cells. *J. Infect. Dis.* 8: 348-360, 1911.
- Bell, P.R. Negative-Capacity Amplifier, App. A. of M.I.T. Radiation Laboratory, Series 19: 1949(A) McGraw Hill Publishers, New York.
- Bell, P.R. Cathode Compensated Amplifier, *Ibid.* App. B, 1949 (B).
- Bode, H.W. Network Analysis and Feedback Amplifier Design. D. Van Nostrand Company, Inc., New York. 1945.
- Brock, L.G., Coombs, J.S., and Eccles, J.C. Action potentials of motor neurones with intracellular electrodes. *Proc. Univ. of Otago Med. Sch.* 29: 14-15, 1951.
- Chambers, R. New apparatus and methods for the dissection and injection of living cells. *Anat. Rec.* 24: 1-19, 1922.
- Curtis, H.J. and Cole, Kenneth S. Membrane action potentials from the squid giant axon. *J. Cell. & Comp. Physiol.* 15: 147, 1940.
- Draper, M.H. and Weidmann, S. Cardiac resting and action potentials recorded with an intracellular electrode. *J. Physiol.* 115: 74, 1951.
- Eccles, J.C. Neurophysiological Basis of Mind. Clarendon Press, Oxford, England, 1952.
- Frank, K. Personal communication (1953).
- Graham, J. and Gerard, R.W. Membrane potentials and excitation of impaled single muscle fibers. *J. Cell. & Comp. Physiol.* 28: 99-117, 1946.
- Hartline, H.K., MacNichol, E.F., Jr., and Wagner, H.G. Electrical Responses to Illumination of Isolated Visual Receptor Elements. *Abs. Ann. Meeting of Nat. Acad. Sci., Science* 117: 467, 1953.
- Hodgkin, A.L. and Huxley, A.F. Action potentials recorded from inside a nerve fiber. *Nature* 144: 710, 1939.
- Krakauer, S. Electrometer triode follower. *Rev. Sci. Instr.* 24: 496-500, 1953.
- Ling, G. and Gerard, R.W. The membrane potential and metabolism of muscle fibers. *J. Cell. & Comp. Physiol.* 34: 413, 1949.
- MacNichol, E.F., Wagner, H.G., and Hartline, H.K. Electrical Activity Recorded Within Single Gnatidia of the Eye of *Limulus*. *Abs. XIX International Physiological Congress*, Montreal, Canada, 1953.
- Nastuk, W.L. and Hodgkin, A.L. The electrical activity of single muscle fibers. *J. Cell. & Comp. Physiol.* 35: 39-73, 1950.

- Solms, S.J., Nastuk, W.L., and Alexander, J.T. Development of a high fidelity preamplifier for use in the recording of bioelectric potentials with intracellular electrodes. Rev. Sci. Instr.: 24: 960, 1953.
- Tasaki, I. Nervous Transmission. Charles C. Thomas, Springfield, Illinois, 1953.
- Williams, F.C. Inverse Feedback. Sect. 2.4 of M.I.T. Radiation Laboratory Series. 19: 90, 1949. McGraw Hill Publishers, New York.
- Woodbury, J.W. and Patton, Harry D. Properties of spinal cord elements studied with intracellular ultramicroelectrodes. Fed. Proc. 11(1): 175, 1952.
- Woodbury, L.A., Woodbury, J.W., and Hecht, H.H. Membrane resting and action potentials of single cardiac muscle fibers. Circulation 1: 264-266, 1950.

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